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Comparison of *emm* Typing and Ribotyping with Three Restriction Enzymes To Characterize Clinical Isolates of *Streptococcus pyogenes*

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A total of 336 *Streptococcus pyogenes* isolates recently recovered from patients with pharyngitis from 13 countries were characterized by *emm* typing and riboprinting using an automated Riboprinter (Dupont/Qualicon) based on the patterns produced by three restriction enzymes, EcoRI, PstI, and HindIII. Three enzymes were necessary to increase the discrimination of ribogroups formed by each enzyme. A total of 40 ribogroups and 38 *emm* sequences (not counting allelic variations) were identified. Multilocus sequence typing was performed on a sampling of the isolates, and those results were consistent with those of both *emm* typing and ribotyping. Correlations were observed among all three methods.

The automated Riboprinter has been used to characterize clinical bacterial isolates of various species for epidemiological studies (7). The Riboprinter generates patterns based on restriction enzyme digest and the hybridization of the resulting fragments to the rRNA gene proprietary probe. We have previously used riboprinting for typing of *Streptococcus pneumoniae* clinical isolates; in this study, we describe the use of this method for typing of *Streptococcus pyogenes* (3). The standard enzyme recommended by the manufacturer, EcoRI, produced patterns with very high similarities with band shifts or one-band differences for many known, different strains of *S. pyogenes*, making it less useful for typing. Therefore, additional enzymes were examined to determine which enzymes could generate sufficiently variable patterns to allow differentiation of unrelated isolates.

As *emm* typing, a molecular-based sequencing method that correlates well with the M protein of *S. pyogenes*, became more widely used and standardized, a comparison between ribotyping and *emm* typing was undertaken (2, 5). The Centers for Disease Control and Prevention (CDC) maintains a database (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.html) that allows for accurate identification of *emm* type using established parameters for identification (1). The database contains sequences for all identified *emm* types as well as any allelic variations for those types.

Multilocus sequence typing (MLST) has recently come to the forefront as a method for epidemiological characterization and is an excellent means for epidemiological tracking of bacterial infections (8, 14). Internal sequences of seven housekeeping loci are compared to characterize the isolates, and Enright et al. developed a method for *S. pyogenes* typing (4). MLST can be used on any species of bacteria (14), and a public database is maintained as well as primers and protocols for species for which methods have been established (protocols and databases can be found at http://www.mlst.net). A sample of the isolates was typed by MLST to determine whether a correlation between MLST, *emm* typing, and ribotyping could be noted. Ninety-one isolates, many from the larger ribogroups and/or common *emm* types, were MLST typed.

This study examines the correlation between Riboprinting and *emm* typing methods when used to type 336 *S. pyogenes* clinical isolates from 13 countries that included both macrolide-susceptible and -resistant strains. As mentioned above, a subset of strains was also examined with MLST typing.

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MATERIALS AND METHODS

S. *pyogenes* strains were obtained from patients with pharyngitis and were isolated from 13 countries between 2000 and 2002. The isolates were acquired as follows: 117 isolates were obtained from five countries in Eastern Europe, 114 isolates were obtained from the United States, 80 isolates came from four countries in Western Europe, 15 isolates were received from South Africa, 8 isolates came from Israel, and 2 isolates were from Argentina. Isolates were incubated for 18 to 24 h on tryptic soy agar-blood agar at 35°C in 5% CO_2 and were used for susceptibility testing, riboprinting, *emm* typing, and MLST. Susceptibility testing was performed by broth microdilution according to NCCLS guidelines (11).

emm typing was accomplished by using a modified method described by Beall et al. and the CDC website at http://www.cdc.gov/ncidod/biotech/strep/protocols.htm (2). Crude lysates were prepared by suspending cells into 100 μl of water with an inoculating loop and heating them to 95°C for 15 min. For most strains, crude lysates were sufficient, but there were a few strains that required standard DNA preparations in order to obtain PCR product that would yield good sequence (6, 9). The primers and cycling parameters were as described previously except that increasing the annealing temperatures to 50 and 53°C assisted in obtaining good PCR product for a few strains for which 46°C was ineffective. Platinum Supermix and Master Mix, used for *emm* typing and MLST, respectively, were purchased from Invitrogen (Carlsbad, Calif.) and Sigma (St. Louis, Mo.). Primers for PCR and sequencing were purchased from Sigma and/or IDT (Coralville, Iowa).

MLST was done with 91 isolates for comparison. The protocol for *S. pyogenes* was used according to the MLST website (http://www.mlst.net). Crude lysates were prepared as described above for *emm* typing and used as the templates. Isolates from large ribogroups or *emm* type combinations were selected to determine if the MLST sequence type (ST) would be the same. Isolates in the

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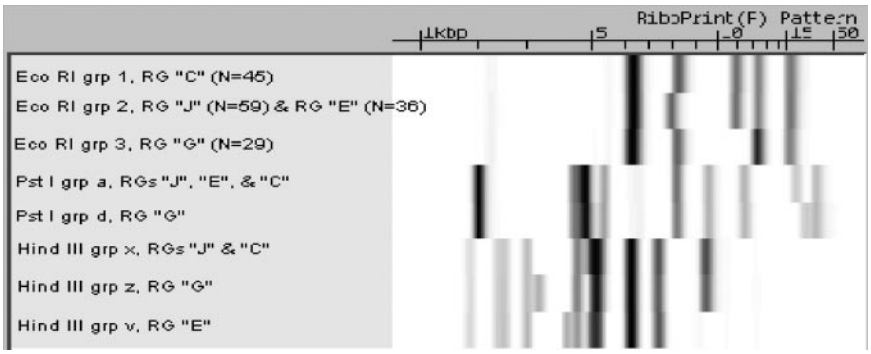

FIG. 1. Examples of riboprint patterns. Comparison of EcoRI, PstI, and HindIII riboprint patterns for common ribogroups is shown.

same ribogroup but with different *emm* types or with the same *emm* type but different ribogroups were selected to establish whether an ST correlation could be established more often with *emm* type or ribogroup.

For ribotyping, we decided upon a protocol using three restriction enzymes, EcoRI, PstI, and Hind III, to differentiate and characterize the 336 isolates discussed here. Eight isolates could be evaluated per run with up to four concurrent runs per 8-h day. Results were available 8 to 10 h from the "start time" on the unit. A 1-μl loop of cells was suspended in 200 μl of riboprinter buffer, washed, and resuspended in 200 μl of fresh riboprinter buffer. Thirty microliters of cell suspension was used for Riboprinter analysis after heating was done according to the manufacturer's protocol. The same cell suspension for each isolate was used for the three different restriction enzyme digestions (3). A dendrogram was generated by using the resulting riboprint patterns from the three restriction enzymes using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). All Riboprinter consumables except for HindIII and PstI (which were purchased from New England Biolabs, Beverly, Mass.) were purchased from Dupont/Qualicon (Wilmington, Del.).

Macrolide- and/or lincosamide-resistant isolates were tested for mechanisms of resistance by PCR as described previously (13). The strains were examined for the following mechanisms: *erm*(A) or *erm*(TR), *erm*(B), *erm*(C), and *mef*(A); if no common mechanism was identified, the rRNA, L4, and L22 genes for the isolate were sequenced.

RESULTS

For *S. pyogenes* ribogroup determination, one enzyme alone could not discriminate between isolates adequately because many patterns were highly related, differing only in the position of one band. We found that forming combined ribogroups based on the individual patterns generated by three enzymes was useful in discriminating between isolates. Riboprinting yielded 18 EcoRI types, 28 PstI types, and 14 HindIII types; the three restriction fragment length polymorphism types were combined into a single ribogroup designation. Forty ribogroups were identified based on the riboprint patterns from the three enzymes. Figure 1 shows the riboprint patterns for the four largest ribogroups which contain 50% (169) of the isolates studied. Many combined ribogroups differed in only one enzyme pattern. Ribogroups correlated well with *emm* typing, and groups that differed by a single band often had different *emm* types. Eight ribogroups contained 68% (231) of the isolates. Thirty-eight *emm* types were identified, not counting allelic variations. Approximately 70% (237) of the isolates evaluated were found among nine *emm* types.

The most represented ribogroups among these isolates were ribogroup J (59 isolates with the following *emm* types: *emm* type 28 [*emm* 28] [49 isolates], *emm* 44/61 [7 isolates], *emm* 76 [1 isolate], and *emm* 82 [2 isolates]), ribogroup C (45 isolates with the following *emm* types: *emm* 77 [34 isolates], *emm* 2 [6 isolates], *emm* 68 [2 isolates], *emm* 75 [2 isolates], and *emm* 8 [1 isolate]), ribogroup E (36 isolates with the following *emm* types: *emm* 12 [35 isolates] and *emm* 75 [1 isolate]), and ribogroup G (29 isolates with the following *emm* types: *emm* 1 [26 isolates], *emm* 3 [1 isolate], *emm* 80 [1 isolate], and one *emm* similar to *st*1207). Allelic variation of *emm* types 3, 5, 22, 28, 58, 81, and 89 were observed (data not shown). Table 1 summarizes the ribogroups found in this study with associated *emm* types.

Table 2 summarizes the ribogroups and *emm* types associated with the macrolide-resistant isolates and their countries of origin. Eighty-nine isolates were macrolide resistant, 30 iso-

TABLE 1. Ribogroups[a]

Ribogroup	Total no. of isolates	% of total isolates	*emm* type(s) (no. of isolates)
J	59	17.5	28 (49), 44/61 (7), 76 (1), 82 (2)
C	45	13.4	77 (34), 2 (6), 68 (2), 75 (2), 8 (1)
E	36	10.7	12 (35), 75 (1)
G	29	8.6	1 (26), 3 (1), 80 (1), similar to *st*1207 (1)
D	18	5.4	4 (18)
V	17	4.8	75 (17)
H	14	4.1	89 (14)
L	13	3.9	6 (13)
T	10	3	58 (8), 106 (1), 77 (1)
F	9	2.7	5 (9)
A	9	2.7	3 (9)
D1	8	2.4	87 (8)
Q	7	2.1	11 (7)
W	5	1.5	58 (5)
S	5	1.5	73 (5)
F1	5	1.5	78 (5)
U	5	1.5	81 (5)
P	4	1.2	12 (4)
Y	4	1.2	22 (4)
X	4	1.2	77 (3), 2 (1)
N	3	0.9	94 (3)
B	2	0.6	44/61 (2)
Z	2	0.6	1 (1), similar to *st*1207 (1)
S1	2	0.6	6 (2)
C1	2	0.6	28 (2)
K	2	0.6	18 (2)
M	2	0.6	118 (2)

[a] The following ribogroups each have only one isolate (these 15 groups account for 4.4% of the isolates): O1 and P1(*emm* 9), O and I (*emm* 1), A1 (*emm* 95), E1 (*emm* 92), H1 and L1 (*emm* 102), Q1 (*emm* 41), K1 (*emm* 50), M1 (*emm* 60), N1 (*emm* 31), G1 (*emm* 95), R (*emm* 22), and B1 (*emm* 74).

TABLE 2. Macrolide-resistant isolates: ribogroup, *emm* type, and country of orgin

Resistance mechanism	No. of isolates	Ribogroup	*emm* type	No. of isolates per ribogroup-*emm* type	country (no. of isolates)[a]
erm(B)	34	J	28	23	Fr (12), H (7), C (4)
		Q	11	2	Fr (1), GB (1)
		S	73	2	U (2)
		C	68	1	U
		C1	28	1	Fr
		E1	92	1	U
		H	89	1	G
		J	76	1	U
		M1	60	1	H
		V	75	1	U
erm(A)	30	C	77	8	P (6), Fi (2)
		J	44/61	5	P (5)
		T	58	5	U (5)
		W	58	5	U (3), Fr (1), H (1)
		V	75	3	P (3)
		N	94	2	U (2)
		E	12	1	U
		Q	11	1	C
mef(A)	24	D	4	8	G (3), S (3), Fr (1), H (1)
		E	12	8	U (4), H (2), P (1), S (1)
		C	75	2	U (2)
		P	12	2	H (1), U (1)
		A1	95	1	S
		G	1	1	Fr
		H1	102	1	GB
		J	82	1	GB
23S mutant	1	E	12	1	P

[a] C, Czech Republic; Fi, Finland; Fr, France; G, Germany; GB, Great Britain; H, Hungary; P, Poland; S, South Africa; U, United States.

lates were *erm*(A) or *erm*(TR), 34 isolates were *erm*(B), 24 isolates were *mef*(A), and 1 isolate had two 23S ribosomal mutations: G2057A and A2059G substitutions in the rRNA gene. *erm*(A) occurred in eight different ribogroups and seven different *emm* types. *erm*(A) was most frequently identified in ribogroup C, *emm* 77; ribogroup J, *emm* 44/61; ribogroup T, *emm* 58; and ribogroup W, *emm* 58, which accounted for 76% of the *erm*(A) isolates. Five *erm*(A) isolates were macrolide and lincosamide resistant; three of these isolates were ribogroup V, *emm* 75, and all three were isolated in Poland. *erm*(B) was identified in 10 different ribogroups and nine *emm* types. Twenty-three of the *erm*(B) isolates were ribogroup J, *emm* 28, and all the isolates in this group came from Europe; these isolates accounted for 68% of the *erm*(B) isolates identified. *mef*(A) was identified in eight ribogroups and seven *emm* types but was most frequently identified in ribogroup D, *emm* 4, and ribogroup E, *emm* 12. Ribogroup D, *emm* 4, and ribogroup E, *emm* 12, accounted for 66% of the *mef*(A) isolates.

MLST typing was done on 91 isolates. Isolates were selected based on ribogroup and/or *emm* type to compare similarities or differences between the most common *emm* types and ribogroups. Table 3 summarizes the MLST results. In general, if isolates were in the same ribogroup and had the same *emm* type, the ST was also the same. For example, all 10 of the ribogroup C *emm* 77 isolates selected for MLST had the same ST. Nine of the 11 ribogroup J *emm* 28 isolates were the same ST, while the other 2, both isolated in Finland, differed in the *yqiL* allele but showed 99% similarity to allele 19, differing in only one base. Strains with differences at one or two loci are considered variants and closely related by MLST (4). Interestingly, neither of the two ribogroup J *emm* 28 isolates with the slightly different *yqiL* allele harbored *erm*(B) as did others in ribogroup J, *emm* 28. However, if the *emm* types and ribotypes did not concur, it was difficult to see a correlation to ST. For example, isolates in ribogroup E, *emm* 12, and ribogroup E, *emm* 75, had very similar STs (differing only at one base in one locus), while ribogroup C had five associated *emm* types, all with very different ST types (Table 1). All 13 of the isolates in ribogroups T and W, *emm* 58, were found to be ST 176, an ST recently identified by McGregor et al. (10). Ten of these strains (five of ribogroup T and five of ribogroup W) were macrolide resistant, harboring *erm*(A) (Table 2). Ribogroup J, *emm* 44/61, contained four isolates that had the same single base change in allele *yqiL* compared to ST 25. All four were isolated in Poland, and three were found to harbor *erm*(A).

A number of isolates with the same *emm* type and ST were in different but related ribogroups; ribogroups T and W (*emm* 58, ST 176), E and P (*emm* 12, ST 36), J and C1 (*emm* 28, ST 52), C and X (*emm* 73, ST 63), C and X (*emm* 2, ST 58), G and Z (*emm* 1, ST 28), and G and Z (*emm* similar to *st*1207, ST 28) all had isolates with the same *emm* types and STs (MLST types). Examination of the riboprint patterns revealed that ribogroups G/Z, C/X, E/P, J/C1, and T/W all showed similar changes in riboprint patterns. Ribogroups Z, X, P, C1, and W

TABLE 3. Comparison of ribogroup, *emm* type, and MLST results

Ribogroup	*emm* type	No.[a]	Locus sequence type[b]							Sequence type[c]	Nearest neighbor[d]
			gkl	*gtr*	*mur*I	*mut*S	*recP*	*xpt*	*yqiL*		
G	1	6	4	3	4	4	4	2	4	28	
I	1	1	4	3	4	4	4	2	4	28	
O	1	1	6	5	7	1	5	6	3	42	
Z	1	1	4	3	4	4	4	2	4	28	
C	2	2	11	9	1	9	2	3	4	55	
X	2	1	11	9	1	9	2	3	4	55	
A	3	1	2	6	8	5	2	3	2	15	
A	3	1	2	6	8	5	2[e] (92T)	3	2	15[e]	
A	3	1	2	29	8	5	2	3	2		15 (6) or 18 (6)
G	3	1	2	6	8	5	2	3	2	15	
D	4	3	5	11	8	5	15	2	1	39	
C	8	1	51	3	8	25	33	3	27		110 (5) or 165 (5)
E	12	12	5	2	2	6	6	2	2	36	
P	12	2	5	2	2	6	6	2	2	36	
C1	28	2	11	6	14	5	9	17	19	52	
J	28	9	11	6	14	5	9	17	19	52	
J	28	2	11	6	14	5	9	17	19[e] (427A)	52[e]	
T	58	8	3	2	3	3	1	3	3	176	
W	58	5	3	2	3	3	1	3	3	176	
B1	74	1	92	2	2	2	31	3	2		
C	75	1	11	2	1	3	12	3	7	49	
E	75	1	5	2	2[e](267G)	6	6	2	2	36[a]	
V	75	2	11	2	1	3	50	8	7	150	
J	76	1	11	6	3	6	6	27	4	50	
C	77	10	13	6	2	3	23	3	11	63	
T	77	1	4	31	2	11	34	3	21		35 (6), 166 (6), or 347 (6)
X	77	1	13	6	2	3	23	3	11	63	
G	80	1	11	2	14[e](84A,96C)	3	9[e](36T)	13	1[e](240G)		
J	82	1	4	65	21	16	17	13	1		26 (6)
N	94	2	24	2	3	5	1	3	1	89	
G	Similar to *st*1207	1	4	3	4	4	4	2	4	28	
Z	Similar to *st*1207	1	4	3	4	4	4	2	4	28	
B	44/61	1	4	2	3	11	17	3	1	25	
J	44/61	4	4	2	3	11	17	3	61	367	
J	44/61	1	4	2	3	11	17	3	1	25	
C	68-1	1	11	9	1	7	2	8	3		

[a] Number of isolates typed in each group.
[b] The seven loci used in MLST for *S. pyogenes.*
[c] Sequence type assigned for seven types.
[d] Nearest sequence type (number of alleles matched if ≥5) if profile is not in database at http://www.mist.net. Strains are considered genetically realated if they share five or six loci.
[e] Indicates >99% similarity to listed allele if no new allele number has been assigned for the observed sequence. The variant base number(s) and change(s) are in parentheses. MLST allele numbers, sequence type, and variant bases were obtained from http://www.mlst.net.

all lacked the same single band in the PstI and HindIII patterns relative to ribogroups G, C, E, J, and T. Their respective EcoRI groups and patterns remained the same for each pair (Fig. 2 and Table 3).

In summary, there were eight examples (involving 64 isolates) of the same ST among isolates that were the same ribogroup and *emm* type. There were three examples of the same ribogroup and *emm* type showing slightly different but closely related STs. Among those that were in the same ribogroup but with different *emm* types, there were only two examples of the same ST (ribogroups G and Z both contained isolates with *emm* 1 and an *emm* similar to *st*1207, and all were ST 28). Among those that were found to have the same *emm* type but different ribogroups, there were eight examples of the same ST type and eight examples of different ST types, although three of those differed by only one base in ST, and strains that vary at one or two MLST loci are considered closely related (4).

DISCUSSION

From the 336 isolates, 40 ribogroups were identified and 38 *emm* sequences were obtained (not counting allelic variations). Ribogroups often had more than one associated *emm* type; of the 21 ribogroups containing more than one isolate, 7 had more than one associated *emm* type. Usually, there was a predominant *emm* type within the ribogroup. The largest of the ribogroups, J, C, E, and G, were found to have 83, 75, 97, and 87% of their isolates with the same *emm* type, respectively. The isolates from seven ribogroups were further differentiated by *emm* typing, while the isolates from 14 *emm* types were further characterized by riboprinting.

A dendrogram comparing some of the ribogroups was generated to establish the relatedness of a sample of the combined groups (Fig. 2). The dendrogram was generated by using the composite of all three patterns produced by the three enzymes

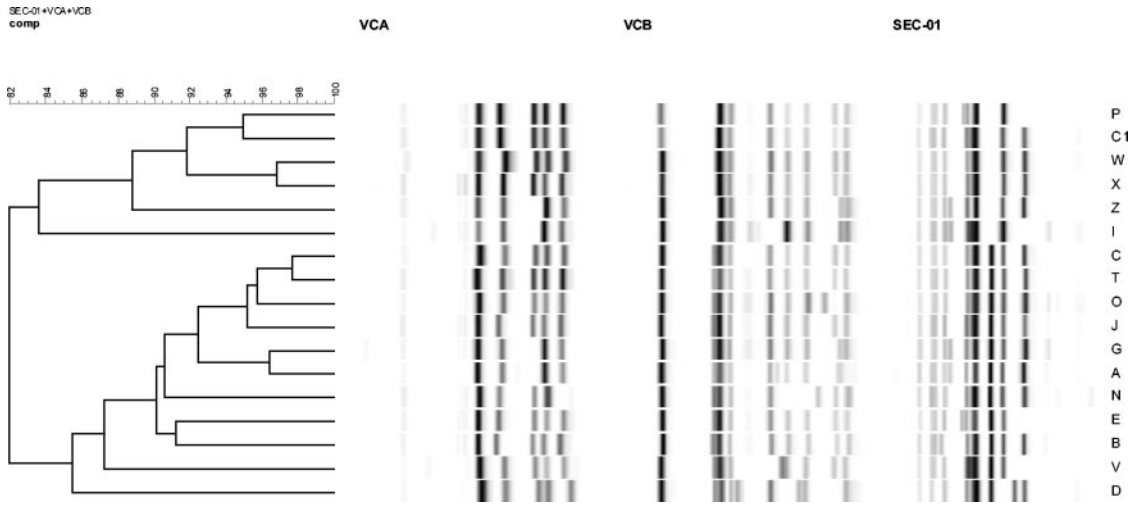

FIG. 2. Dendrogram of ribogroups P, C1, W, X, Z, I, C, T, O, J, G, A, N, E, B, V, and D are shown as representative groups. VCA shows the EcoRI pattern, and VCB and SEC-01 show PstI and HindIII patterns, respectively. The dendrogram is a composite of all three patterns for each ribogroup.

used for riboprint characterization. Ribogroups C and T were 97% related, but there was only one isolate in ribogroup T that had a common *emm* type with the isolates in ribogroup C. Ribogroups J and C were 95% related, yet there were no examples of the same *emm* type between these two groups. Ribogroups G and A were 96% related, but only one isolate in ribogroup G had the same *emm* type as those in ribogroup A. Conversely, ribogroups T and W were <85% related, yet the majority of isolates in ribogroup T and all the isolates in ribogroup W were *emm* 58 and ST 176.

In general, results of MLST concurred with those of ribotyping and *emm* typing; if the isolates were in the same ribogroup and had the same *emm* type, the MLST typing was the same (or nearly the same, i.e., 99% similar) at all seven loci (strains that vary at one or two loci are considered related [4]). This observation was not surprising since the majority of these isolates were collected from Western Europe, Eastern Europe, and the United States and is consistent with the analysis by McGregor et al. which showed that group A streptococcal disease in developed countries is caused by a small number of clones (10). This analysis is further supported by the observation that approximately 70% of the isolates studied were contained in 20% of the ribogroups and 24% of the *emm* types identified here.

Among the isolates selected for MLST, there are 17 examples of further characterization by riboprinting among eight *emm*-ST combinations (see Results) (Table 3 and Fig. 2). Examination of some of the riboprint patterns of related groups with the same ST and *emm* type revealed that ribogroups G/Z, C/X, E/P, J/C1, and T/W all showed similar changes in riboprint patterns (Fig. 2). The number of isolates found in ribogroups Z, X, P, C1, and W ($n = 17$) were relatively small compared to the number of isolates in ribogroups G, C, E, J, and T ($n = 158$). Perhaps these were strain variants, particularly since such a small number of isolates were involved compared to the large ribogroups to which they are related. Clonal spread of isolates should also be considered since all the isolates in ribogroup W were *erm*(A) and ribogroup W contained the most (five) isolates of any group showing the differences in riboprint patterns discussed. The clinical significance of further discrimination of isolates with the same *emm* type and ST by riboprinting has not been determined.

Macrolide resistance occurred in many different ribogroups and *emm* types. However, 79% of the ribogroup J *emm* 28 isolates from France (12 of 12), Hungary (7 of 11), and the Czech Republic (4 of 6) harbored *erm*(B); there were 20 ribogroup J *emm* 28 isolates from the United States (11 isolates), Finland (6 isolates), Great Britain, Poland, and South Africa (1 isolate each), but none were found to be macrolide resistant.

Many of the macrolide-resistant isolates identified here had the same *emm* type and ST as those identified in Germany by Reinert et al., although our isolates were from countries other than Germany, as shown in Table 2 (12). We found, as reported by Reinert et al., *mef*(A) isolates that were *emm* 1, ST 28 (ribogroup G [France]); *emm* 4, ST 39 (ribogroup D [South Africa]); *emm* 12, ST 36 (ribogroups E [United States] and P [Hungary]); and *emm* 75, ST 49 (ribogroup C [United States]). Similarly, we also identified *erm*(A) isolates with *emm* 77, ST 63 (ribogroup C [Poland and Finland]), and *emm* 44/61 (ribogroup J [Poland]). Our *erm*(A) *emm* 44/61 isolates appear to be closely related to strain 380 identified by Reinert et al. based on the *yqiL* allele variation. However, there were no *erm*(B) isolates identified in this study that had the same *emm* type (*emm* types 1, 12, 22, or 77) as those identified by Reinert et al. (12). Thus, there appears to be evidence not only for clonal spread of isolates carrying macrolide-resistant determinants [*erm*(B), ribogroup J, *emm* 28] in Europe but also for the mobility of these elements, since the resistance mechanisms were found in a large number of different strain types.

In summary, 336 clinical isolates of *S. pyogenes* from patients with pharyngitis were analyzed by *emm* typing and ribotyping using an automated Riboprinter. *emm* typing is an established method that characterizes *S. pyogenes* by examining the DNA sequence of the M protein that determines the surface antigens on the cell. Beall et al. (2) have characterized many types and have generated an extensive public database maintained by the

CDC. The advantages to using *emm* typing include a direct comparison of isolates studied by different laboratories and the ability to track the prevalent types of invasive-disease-causing strains. MLST is quickly becoming the most common method for bacterial epidemiological characterization with an extensive, growing public database. MLST is a very useful method for the characterization of bacterial isolates because it considers seven loci in the analysis. Ribotyping with the Riboprinter is an automated process which requires little preparation and has a rapid turnaround time. Ribotyping is a useful, consistent method that allows easy comparison of isolates studied at the same facility. The database allows for comparison of patterns generated from all the isolates that have been studied with the unit. Since the database is unique and is maintained in each individual Riboprinter unit, riboprinting comparisons between laboratories would not be as easy or as useful as *emm* typing or MLST for broad-scale typing and tracking. A correlation was noted between *emm* typing and riboprinting, and a correlation was also noted between *emm* typing, riboprinting, and MLST typing. When isolates were found to have both the same *emm* type and ribogroup, they usually had the same ST. MLST is the most powerful of the three methods described here for discriminating between isolates, although there were examples of riboprinting discriminating between isolates that had the same ST and *emm* type; however, the clinical significance of this discrimination is not known. Ribotyping coupled with *emm* typing was found to be an excellent method for the efficient characterization of different isolates of *S. pyogenes* at our laboratory.

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